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Dopamine genes and attention-deficit hyperactivity disorder: a review

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Objective: To review the results of genetic studies investigating dopamine-related genes in attention-deficit hyperactivity disorder (ADHD). **Data sources:** Papers (association/linkage, meta-analyses and animal model studies) were identified through searches of the PubMed database and systematically reviewed. **Data synthesis:** Consistent results from molecular genetic studies are pointing strongly to the possible link between 2 specific genes, the dopamine transporter (*SLC3A6*) and the dopamine receptor 4 (*DRD4*), and ADHD. **Conclusions:** The implication of *SLC6A3* and *DRD4* genes in ADHD appears to be one of the most replicated in psychiatric genetics and strongly suggests the involvement of the brain dopamine systems in the pathogenesis of ADHD. However, more work is required to further these findings by genotype-to-phenotype correlations and identify the functional allelic variants/mutations that are responsible for these associations. The role of other dopamine genes, which may have smaller effects than *SLC6A3* and *DRD4*, needs also to be determined.

Objectif : Examiner les résultats d'études en génétique portant sur les gènes liés à la dopamine chez les personnes atteintes du trouble d'hyperactivité avec déficit de l'attention (THADA). **Sources des données :** Des documents (études d'association ou de lien, méta-analyses et études sur modèle animal) recensés grâce aux recherches effectuées dans la base de données PubMed ont fait l'objet d'un examen systématique. **Synthèse des données :** Des résultats homogènes provenant d'études en génétique moléculaire indiquent nettement la possibilité d'un lien entre le THADA et deux gènes précis, soit le transporteur de la dopamine (*SLC3A6*) et le récepteur D4 de la dopamine (*DRD4*). **Conclusions :** Il semble que le lien entre les gènes *SLC6A3* et *DRD4* et le THADA soit l'un des plus souvent établis dans le domaine de la génétique en psychiatrie. Cela porte fort à penser que les systèmes de dopamine du cerveau interviennent dans la pathogenèse du THADA. Il faudra toutefois effectuer d'autres travaux afin de préciser ces constatations selon les corrélations génotype-phénotype et de cerner les mutations ou allèles fonctionnels qui causent ces associations. Il faut aussi déterminer le rôle d'autres gènes de la dopamine, dont les effets pourraient être moins importants que ceux des gènes *SLC6A3* et *DRD4*.

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Medical subject headings: alleles; attention deficit disorder with hyperactivity; case-control studies; dopamine; dopamine beta-hydroxylase; genes; genetic predisposition to disease; meta-analysis; minisatellite repeats; molecular biology; polymorphism, genetics; receptors, dopamine.

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Introduction

Attention-deficit hyperactivity disorder (ADHD) is a childhood onset, clinically heterogeneous disorder characterized by excessive motor activity, impulsiveness and inattention. Roughly 5%–10% of all school-aged children worldwide have ADHD,[1-4] and it is not uncommon for the condition to persist into adulthood.[5] Although the etiology of ADHD is unknown, family, twin and adoption studies have demonstrated high familiality[6-8] due mainly to shared gene effects.[9] It is widely accepted that several genes, each contributing a small fraction of the total genetic variance, are implicated in ADHD.[10,11]

Several lines of evidence indicate dopamine system dysfunction in the pathogenesis of ADHD. First, methylphenidate, amphetamine and other psychostimulant drugs that inhibit the activity of the dopamine transporter and increase synaptic levels of dopamine effectively control ADHD symptoms. Second, magnetic resonance imaging and single-photon emission computerized tomography studies[12] demonstrate abnormalities in neuroanatomical areas with rich dopamine innervations in ADHD children. Third, animal studies strongly suggest that abnormalities of dopamine neurotransmission may be pivotal in motor control[13-16] and other neuropsychological[17] functions purportedly affected in ADHD.

Recently, polymorphic sites at dopamine-related genes — encoding for enzymes, receptors and transporters, many of which cause observed alterations in protein function or structure — have been identified, prompting researchers to test their role in increasing the risk for ADHD. The main objective of this paper is to review the results of studies investigating dopamine-related genes in ADHD. We will review data relating each gene to ADHD or its major symptoms and summarize the literature specifically devoted to investigating the risk conferred by various alleles of the gene to the development of ADHD.

Methods

The search for susceptibility genes of small effect in a polygenic disorder such as ADHD has been approached in a number of ways. In contrast to many other psychiatric disorders, there were very few linkage studies in ADHD. Indeed, the only genome-wide scan for susceptibility loci among ADHD-affected sibling pairs was published recently by the group of Smalley.[11] In this study, loci conferring a substantial amount of risk to develop ADHD in siblings of affected individuals (relative risk ≥ 2.5 as compared with the risk in the general population) were undetectable in 92% of the human genome, curtailing the possibility of a major susceptibility gene in ADHD. Only one other study investigating markers in the 20p11-p12 locus, syntenic to the mice 2q locus deleted in the coloboma mice model of ADHD, was published. No linkage was identified between ADHD and markers in this locus.[18] Comings' extensive review of the molecular genetics of ADHD in 2001[19] showed further evidence of polygenicity and limited variance explained by each gene implicated in the disorder.

Case–control association studies comparing frequencies of marker alleles in ADHD patients to those in unrelated control subjects are numerous. Data based on this type of analysis, however, are often difficult to interpret because of the possibility of population stratification, namely that ethnic differences in allele frequencies can contribute to observed differences between affected subjects and controls. Family-based association designs, in which parental or full sibling genotypes are used as "internal controls,"[20] are favoured because they control for outside sources of variance, including ethnic variance in allele frequencies. Several statistics have been proposed to test for association between an allele in a candidate gene and a disease, including the haplotype-based haplotype relative risk (HHRR) test, in which alleles transmitted to affected children are compared with alleles that are not transmitted.[21] Another test, the transmission disequilibrium test (TDT), is currently the most robust test for "linkage" with association and is designed to control for population subdivision and admixture,[22] although the TDT may be statistically less powerful[23] and may result in some selection bias[24] compared with the population-based case–control association design.

Papers included in this review were identified by searching journal abstracts available online through PubMed at the National Library of Medicine using a number of search keywords for each of the candidate genes, including: "association studies," "meta-analyses," "animal model" and the specific name of the gene (e.g., "DRD3" or "dopamine receptor 3"). Relevant papers that were not listed in the PubMed database but that we identified while reviewing papers listed in PubMed were also reviewed. We limited our search for papers published to English-language papers.

Results

Dopamine transporter gene (SLC6A3)

The dopamine transporter gene (*SLC6A3*) is of great interest given that methylphenidate is theorized to inhibit the function of this transporter by preventing presynaptic reuptake of dopamine. Giros et al[13] developed a dopamine transporter knock-out (*Slc6a3*-KO) mouse, which displayed behavioural traits highly reminiscent of ADHD characteristics observed in humans. Indeed, *Slc6a3*-KO mice were spontaneously hyperactive, had higher levels of motor activity induced by stress compared with wild-type animals and were significantly calmed by the administration of amphetamine or methylphenidate. In addition, dopamine was found to remain 100 times longer in the extracellular medium of homozygous *Slc6a3*-KO mice than in heterozygous and wild-type animals.

The human (*SLC6A3*) gene was localized by Giros et al[25] and Vandenbergh et al[26] to chromosome 5p15.3. Sequence analysis of this gene revealed a VNTR (variable number of tandem repeats) polymorphism with a 40-bp unit repeat length, ranging from 3 to 11 copies.

Published association and linkage studies of the *SLC6A3* gene in ADHD humans are indicated in Table 1; all focused primarily on the 3′ VNTR marker, in particular the 10-repeat (480-bp) putative high-risk allele, as well as the 9-repeat 440-bp allele. All of the studies investigated an association between *SLC6A3* and ADHD using either the TDT or the HHRR test. Interestingly, only 1 of 6 groups using the TDT identified linkage compared with 3 of 4 groups who applied HHRR analysis. Although the underlying reason for such a discrepancy is largely unknown, linkage in the TDT studies may be difficult to detect if sample sizes are insufficient for each group. In addition to studies included in Table 1, Todd et al[35] examined association using the TDT in a population sample of twins. They found no significant disequilibrium of the VNTR alleles using a number of ADHD diagnostic systems.

Curran et al[29] recently combined available data from published studies of the VNTR polymorphism and, using the TDT, found evidence for association and linkage (odds ratio = 1.15, p = 0.06). Similarly, Swanson et al[36] combined the *SLC6A3* data from 3 earlier studies to measure allele proportions of the 10-repeat VNTR polymorphism among ADHD populations. Using the HHRR method, a significantly greater frequency of the 10-repeat allele was observed compared with control groups, indicating that the transporter gene is likely to be implicated in the etiology of ADHD. Notwithstanding, other studies have suggested a limited association between the VNTR polymorphism and *SLC6A3* expression in humans.[37] More studies with larger samples will be needed to further elucidate the role of *SLC6A3* in ADHD.

Taking an interesting pharmacogenetic approach, Winsberg and Comings[38] have also reported that homozygosity for the 10-repeat allele of the *SLC6A3* was significantly increased in African-American children with ADHD symptoms who respond poorly to methylphenidate. Although promising, the results of this study should be considered cautiously because of several limitations discussed by the authors, including the fact that the assessment of therapeutic response to methylphenidate was based on an open trial.

Table 1: Studies of the association between attention-deficit hyperactivity disorder (ADHD) and the *SLC6A3* 480-bp VNTR allele

Study	Location	No. of probands	Diagnostic system	Test of association	Linkage	Statistic	p value
Barr et al[27]	Canada	102	DSM-IV	TDT	–	χ^2 = 2.6	0.06
Roman et al[28]	Brazil	81	DSM-IV	HHRR	–	χ^2 = 0.02	0.88
Curran et al[29]	Turkey	111	DSM-IV	TDT	–	χ^2 = 0.93	0.34
Curran et al[29]	United Kingdom	66	DSM-IV	TDT	+	χ^2 = 8.97	0.001
Holmes et al[30]	United Kingdom	137	ICD-10, DSM-IV and DSM-III-R	TDT	–	OR = 0.89	0.59
Palmer et al[31]	United States	209	DSM-IV and DSM-III-R	TDT	–	OR = 0.88	0.40
Daly et al[32]	Ireland	118	DSM-IV	HHRR	+	RR = 1.2	0.006
Waldman et al[33]	United States	122	DSM-IV	TDT	+*	OR = 1.63	0.06
Cook et al[34]	United States	49	DSM-III-R	HHRR	+	OR = 3.17	0.01

Note: DSM = *Diagnostic and Statistical Manual of Mental Disorders*; TDT = transmission disequilibrium test; HHRR = haplotype-based haplotype relative risk; OR = odds ratio; ICD-10 = *International Statistical Classification of Diseases and Related Health Problems, Tenth revision*; RR = relative risk.
*For combined type only.

Dopamine receptor 1 (DRD1)

Xu et al[14] found that *DRD1* mutant mice exhibited heightened locomotor activity and did not respond to dopamine agonists (SKF81297) and antagonists (SCH23390), indicating that a nonaltered functioning of D_1 receptors is critical for the expression of normal motor activity. A more recent study with rats[15] suggests that D_1 receptors in the prefrontal cortex may be involved in modulating attentional function, but this study has yet to be replicated. Additionally, Goldman-Racik's group[17] reported an association between D_1 receptors in the prefrontal cortex and deficits in working memory, an executive function that has been studied and previously found to be disturbed in ADHD children.[39]

The only published study of *DRD1* in ADHD[10] did not implicate the receptor in increasing risk for ADHD. Further studies of this and other *DRD1* polymorphisms are needed to expound the gene's involvement in ADHD.

Dopamine receptor 2 (DRD2)

Balk et al[40] used homologous recombination to generate D_2-receptor-deficient mice. These mice displayed reduced locomotor activity, as well as reduced spontaneous movements, analogous to symptoms of Parkinson's disease. Four polymorphic markers have been identified within a 25-kb haplotype system in humans.[41] These markers include 3 *Taq*I restriction site (*Taq*I sites "A," "B" and "D") and 1 short tandem repeat polymorphism. In 1996, Comings et al[42] reported an association between the *A1* allele of the dopamine D_2 receptor gene (*DRD2*) and ADHD as a Tourette's syndrome associated comorbid behaviour. In addition, a relation was found between the severity and accuracy of ADHD diagnosis in subjects with Tourette's syndrome and genetic loading for specific alleles of *DRD2, SLC6A3* and *DBH* genes (in order of relative importance based on correlation [r^2] analysis). In contrast, Rowe et al[43] found that higher counts of ADHD symptoms (based on *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition,* criteria) were associated with decreasing frequencies of the *DRD2*A1* allele. Moreover, a positive correlation was found between the *A2* allele and hyperactive-impulsive symptoms, and less so for the inattentive subtype. However, when parental genotypes were used as controls for population heterogeneity, no significant results were found in Rowe's study. Rowe argues this discrepancy from Comings' results may be the effect of multiple haplotypes with either the *A1* or *A2* alleles in linkage disequilibrium with a functional polymorphism. Other possible explanations include the heterogeneity between the 2 samples and the possibility that these results are false-negative findings. Furthermore, association of ADHD and other behavioural phenotypes with *DRD2* genotypes may depend to a significant degree on environmental exposures such as history of family stress.[44,45] Firm conclusions cannot be reached because of the small samples in both studies; larger samples with ethnically matched unrelated or family member controls are needed to validate (or refute) the authors' findings.

Dopamine receptor 3 (DRD3)

Genetic studies using animal models have shown that the dopamine D_3 receptor gene (*DRD3*) may be involved in regulating locomotor behaviour. Accili et al[16] bred mice lacking functional D_3 receptors using targeted mutagenesis. They reported that *DRD3*$^{-/-}$ mice showed increased locomotor activity compared with heterozygotes. Ekman's et al[46] also observed such a relation in rats using a modified antisense oligodeoxynucleotide targeted against rat *DRD3* mRNA. In both studies, however, the relevance of this increased locomotor behaviour to ADHD was not extensively explored.

The *DRD3* gene has been localized in humans by Le Coniat et al[47] to chromosome 3q13.3. A single base-pair polymorphism within the coding region results in an amino acid substitution (Ser → Gly) at position 9 of the gene's amino terminal.[48] Using a hamster model, Lundstrom and Turpin[49] found that the serine allele has a significantly attenuated affinity for dopamine compared with the glycine allele. This led researchers to examine possible associations between this polymorphism and disorders implicating dopaminergic dysfunction, particularly schizophrenia.[50]

Barr et al[51] conducted a linkage study of 2 polymorphisms of the *DRD3* gene and ADHD: the first (mentioned earlier) alters the recognition site for an endonuclease (*Msc*I) and the other, a polymorphism at intron 5, alters an *Msp*I restriction site. This preliminary study does not, in fact, support any linkage between the Ser9Gly polymorphism on the *DRD3* gene and ADHD using the TDT. Similarly, more recent studies of cohorts of 150 ADHD children by Payton et al[52] and 39 children

by Muglia et al[53] using TDT analysis did not identify an association or linkage. Nonetheless, future studies with larger samples are needed to reveal any link between *DRD3* and ADHD.

*Dopamine receptor 4 (*DRD4*)*

Most molecular genetic studies of *DRD4* and ADHD have focused on a VNTR polymorphism, consisting of a 48-bp repeat unit coding for an amino-acid sequence located in the third cytoplasmic loop of the receptor,[54] thought to be involved in G-protein coupling. Roughly 10 *DRD4* VNTR alleles have been identified in the global human population,[55] the most prevalent being the 4-, 7- and 2-repeat alleles, with global mean allele frequencies of 64.3%, 20.6% and 8.2%, respectively. The 4- and 7-repeat alleles, in particular, show considerable variability across populations, ranging from 0.16 to 0.96 and 0.01 to 0.78, respectively.[55] Of particular interest is the 7-repeat allele, given the low frequency of this allele and the similarly low prevalence of ADHD in Asian populations.[56] Mice lacking the *DRD4* gene have been demonstrated to be supersensitive to ethanol, cocaine and methamphetamine; in these mice, synthesis and clearance of dopamine were elevated in the dorsal striatum.[57] Van Tol et al[44] studied cloned receptor variants of *DRD4* and found different properties between the long (7 repeats) and short (2 and 4 repeats) forms of the receptor with respect to clozapine and spiperone binding. This has prompted researchers to conduct genetic association studies investigating this polymorphism and disorders in which dopamine neurotransmission may be involved.

A considerable number of studies, including both case–control and family-based association studies, have focused on the 7-repeat *DRD4* polymorphism and ADHD (Table 2). An additional 120-bp duplication

Table 2: Studies of the association between ADHD and the *DRD4* 7-repeat allele

Study	Location	No. of probands	Diagnostic system	Test of association	Linkage	Statistic	*p* value*
Case–control association studies							
Mill et al[58]	United Kingdom	132	DSM-IV	χ^2	+	OR = 6.2	0.01
Holmes et al[30]	United Kingdom	129	ICD-10, DSM-IV and DSM-III-R	χ^2	+	OR = 1.9	0.001
Muglia et al[59]	Canada	66	DSM-IV	χ^2	+	OR = 2.5	0.01
Comings et al[60]	United States	52	DSM-III-R and DSM-IV	χ^2	+	χ^2 = 6.64	0.01
Rowe et al[61]	United States	70	DSM-IV	χ^2	+	χ^2 = 5.9	< 0.05
Swanson et al[62]	United States	39	DSM-IV and ICD-10	χ^2	+	χ^2 = 4.65	< 0.035
Castellanos et al[63]	United States	41	DSM-III-R	χ^2	–	χ^2 = 0.06	0.81
La Hoste et al[64]	United States	39	DSM-IV	χ^2	+	OR = 3.0	95% CI = 1.3–7.1
Family-based association studies							
Roman et al[28]	Brazil	81	DSM-IV	HHRR	–	χ^2 = 0.37	0.54
Payton et al[52]	United Kingdom	103	ICD-10, DSM-IV and DSM-III-R	TDT	–	N/A	0.75
Mill et al[58]	United Kingdom	85	DSM-IV	TDT, HHRR	–	N/A	
McCracken et al[65]	United States	371	DSM-IV	TDT	+	χ^2 = 5.4	0.02
Barr et al[66]	Canada	82	DSM-IV	TDT	+	χ^2 = 15.68	< 0.016
Holmes et al[30]	United Kingdom	110	ICD-10, DSM-IV and DSM-III-R	TDT	–	OR = 0.95	95% CI = 0.6–1.5
Kotler et al[67]	Israel	49	DSM-IV	HHRR	–	LR = 7.94	0.16
Muglia et al[59]	Canada	66	DSM-IV	TDT	+	z = 1.41	0.07
Hawi et al[68]	Ireland	78	DSM-IV	HHRR	–	χ^2 = 0.00	0.95
Tahir et al[69]	Turkey	104	DSM-IV	TDT	+	χ^2 = 2.79	0.05
Eisenberg et al[70]	Israel	49	DSM-IV	HHRR	–	χ^2 = 0.14	0.71
Faraone et al[71]	United States	54	DSM-IV	TDT	+	χ^2 = 7.4	0.007
Rowe et al[61]	United States	70	DSM-IV	TDT	–	χ^2 = 0.03	N/A
Smalley et al[72]	United States	129	DSM-III-R and DSM-IV	TDT	+	χ^2 = 4.85	0.03
Swanson et al[62]	United States	52	DSM-IV	HHRR	+	χ^2 = 4.65	< 0.035

Note: CI = confidence interval; LR = likelihood ratio.
*Unless otherwise indicated.

polymorphism identified by Seaman et al[73] has also been the focus of some recent studies.[65,74] Although many of the studies identify an association between the *DRD4* polymorphism and ADHD, a number of other studies do not. Faraone et al[75] recently published a meta-analysis of *DRD4* and ADHD. The data were derived from both family-based data (14 studies, 1665 probands) and case–control studies (8 studies, 1266 children with ADHD and 3068 controls). The odds ratio derived from the case–control studies (which indicates the odds of having the 7-repeat allele among individuals with ADHD in relation to the odds for individuals without ADHD) was 1.9 (95% confidence interval = 1.5–2.2, $p < 0.001$). For family-based studies, the odds ratio (an estimate of the haplotype relative risk, the odds of transmission to individuals with ADHD of the 7-repeat allele in relation to other alleles) was 1.4 (95% confidence interval = 1.1–1.6, $p = 0.02$). This strongly implicates *DRD4* in ADHD, highlighting the putative importance of dopamine in its etiology. The meta-analysis conducted by Faraone et al[75] indicates also that, despite the small risk conferred to individuals by the 7-repeat allele, this allele may play an important role at a population level (population attributable risk percent between 9% and 14%) because of its relatively high population frequency.

Nevertheless, *DRD4* has not been uniformly implicated in all studies of ADHD populations. For example, a recent study by Todd et al[76] examined a population-based sample of twins to establish a link between ADHD latent classes and 2 *DRD4* polymorphisms — the exon 3, 7-repeat allele and the 5′ 120-bp allele. No significant association was found between either polymorphism and the latent classes analyzed.

Molecular genetic association studies have also examined the extent to which individual ADHD traits are affected by certain genes. Novelty seeking and *DRD4* being is a common example (see Paterson et al[77] for review), but a link has not been firmly established. Of great interest is a quantitative trait study by Swanson et al[78] reporting the effects of the 7-repeat allele on specific neuropsychological behaviours believed to be trait markers of ADHD. The tasks selected were designed to probe the anterior cingulate gyrus, right dorsolateral prefrontal cortex and other areas proposed by Posner and Raichle as critical loci in the neuroanatomical network theory of attention.[79] No significant differences between those with the 7-repeat allele and those without were found, suggesting the alleles may identify a subgroup of ADHD but not its cognitive components. However, given the small number of patients included in this study, a false-negative result cannot be ruled out.

Dopamine receptor 5 (DRD5)

Functional analysis of expressed *DRD5* variants[80] has identified at least 6 amino acid substitutions, 2 of which are located in the transmembrane domains and have been associated with decreased D_5 receptor agonist binding affinity. Two research teams independently reported associations between *DRD5* polymorphic loci and ADHD. Daly et al[81] reported the attributable fraction for *DRD5* to be 0.20 in 69 ADHD trios compared with 0.08 and 0.12 for *SLC6A3* and *DBH*, respectively. A follow-up study by Barr et al[82] did not reveal linkage of the 148-bp allele, but significant linkage was observed for the 136- and 146-bp alleles. Regression analysis by Comings et al[10] showed that *DRD5* accounted for 0.64% of the genetic variance of their ADHD population. In Payton et al's family-based study of association between various dopamine genes and ADHD,[52] a trend was identified for preferential transmission of the 148-bp allele, although there were no significant associations found. Conversely, Tahir et al[83] reported a marginal linkage ($\chi^2 = 2.38$, $p = 0.06$) of the *DRD5* polymorphism in their sample of children with ADHD using the TDT test. These studies suggest a possible role for *DRD5* in increasing the risk for ADHD, but they remain difficult to interpret.

Catechol-O-methyltransferase (COMT)

The *COMT* gene has been of recent interest in ADHD given that the COMT enzyme is involved in the metabolic degradation of dopamine, norepinephrine and epinephrine — neurotransmitters proposed to be involved in the etiology of ADHD. Gogos et al[84] studied mice bred with a genetically disrupted *COMT* gene; *COMT*$^{-/-}$ female mice displayed impairment in certain measures of anxiety, whereas male mutants were more aggressive, suggesting a role for *COMT* in areas of emotional and social behaviour in mice.

In humans, *COMT* has been localized to the chromosomal region 22q11.1-q11.2.[85–88] Lachman et al[89] have identified a *COMT* single nucleotide polymorphism variant that causes a Val → Met substitution at amino acid 158 of the membrane-bound form of the enzyme.

Homozygosity for methionine leads to a 3- to 4-fold reduction in *COMT* activity, compared with homozygosity for valine. The *COMT* polymorphism also creates a *Nla*III polymorphism made of 2 alleles designated *COMT*H* ("high") and *COMT*L* ("low" enzyme activity) and encoding for valine and methionine, respectively. Palmatier et al[90] studied the distribution of this polymorphism in various populations and found that the *COMT*L* allele frequency varied significantly across populations, from 0.01 to 0.62.

ADHD symptoms have been observed in children with velo-cardio-facial syndrome (VCFS),[91] a condition associated with hemizygous deletions of the *COMT* gene region. This has spurred interest in possible associations between the *COMT* polymorphic locus and ADHD. In addition, it has been reported that *Nla*III polymorphism may modulate neurocognitive functions,[92,93] including working memory, which is subserved by the prefrontal cortex, a region believed to be one of the brain loci disturbed in ADHD. Table 3 summarizes studies published thus far on the association between *COMT* and ADHD. Eisenburg et al[97] observed an association using the HHRR test between the Val high enzyme activity *COMT* allele and the impulsive-hyperactive type of ADHD. This finding is consistent with the use of monoamine oxidase inhibitors in the treatment in children with ADHD[99] — high *COMT* enzyme activity would make less dopamine and norepinephrine available at the synapse, whereas monoamine oxidase inhibitors increase synaptic availability of these neurotransmitters. Barr et al[98] also independently studied the same *COMT* polymorphism in a larger sample of ADHD probands but found no association between *COMT* and ADHD using the TDT. Several other studies have[96] also refuted any association between the *COMT* polymorphism and ADHD.[52,94,95]

Dopamine beta-hydroxylase (DBH)

Dopamine beta-hydroxylase (*DBH*) is responsible for conversion of dopamine to norepinephrine and is released along with catecholamines from the adrenal medulla and from sympathetic nerve endings. The *DBH* gene is located at chromosome 9q34[100] and has been closely linked to the ABO blood group.[101,102]

DBH polymorphisms have been studied in ADHD populations by Daly et al[81] and Comings et al.[10,42] Daly's group found that the *Taq*I *DBH*A2* allele in the fifth intron was preferentially transmitted to ADHD children 124 times and not transmitted 95 times in 86 trios and 19 parent–proband pairs ($p < 0.05$). In addition, transmission of the allele was stronger among families with at least 1 parent who was retrospectively diagnosed with ADHD (relative risk = 1.49 in familial cases v. 1.20 for nonfamilial cases); however, this difference was not statistically significant. Comings et al[42] have twice investigated the effect of *DBH* in ADHD. In the first study,[42] the prevalence of the *DBH*B1* allele was 73.1% ($p = 0.19$), compared with 60.8% in non-Hispanic Caucasian controls. In the second study,[10] using a multivariate linear regression analysis, *DBH* accounted for 0.56% of the total genetic variance of ADHD, but this was not significant ($p = 0.164$). In addition, a recent family-based study[52] of 104 children with ADHD did not demonstrate an association between *DBH* and ADHD. The latest study, carried out by Roman et al,[103] demonstrated a significant association between the *Taq*I allele and *DBH* in their sample of 88 trios (HHRR test, $\chi^2 = 3.61$, $p = 0.03$).

Although these findings do shed interest on the possible association between *DBH* and ADHD, replication with larger samples is needed to support the association in any working model of ADHD pathophysiology.

Table 3: Studies of association between ADHD and the *catechol-O-methyltransferase* polymorphism

Study	Location	No. of probands	Diagnostic system	Test of association	Linkage	Statistic	p value
Payton et al[52]	United Kingdom	98	ICD-10, DSM-IV and DSM-III-R	TDT	–	—	—
Manor et al[94]	Israel	70	DSM-IV	HHRR	–	LR = 1.74	0.19
Tahir et al[95]	Turkey	72	DSM-IV	TDT	–	$\chi^2 = 0.93$	NS
				HHRR		$\chi^2 = 2.2$	NS
Hawi et al[96]	Ireland	94	DSM-IV	HHRR	–	$\chi^2 = 0.18$	0.67
Eisenberg et al[97]	Israel	48	DSM-IV	HHRR	+	$\chi^2 = 4.72$	0.03
Barr et al[98]	Canada	77	DSM-IV	TDT	–	$\chi^2 = 1.25$	0.26

Note: NS = not significant; for other abbreviations, see foonotes of Tables 1 and 2.

Discussion

A number of theories have postulated the involvement of brain dopamine pathways in the attention and executive functions that are believed to be altered in ADHD. Posner and Raichle's[79] theory of attention involves a neuroanatomical network with a number of areas rich in dopamine innervation, including the prefrontal cortex, cingulate gyrus and anterior basal ganglia. MRI[104] and other imaging techniques[105] have identified abnormalities in these areas in children with ADHD, adding some experimental basis to this theoretical framework implicating dopamine in attention control. The most compelling evidence of the involvement of dopamine in ADHD derives from the fact that dopamine enhancers such as amphetamine and methylphenidate improve behavioural symptoms of ADHD. However, despite these converging lines of evidence implicating brain dopamine circuitry in ADHD, direct and firm evidence of its involvement remains elusive. Remarkably, this difficult and vexing problem is starting to be resolved by genetic studies. Indeed, consistent results from molecular genetic studies are pointing strongly to the possible link between 2 specific genes, *SLC6A3* and *DRD4*, and ADHD.

The *SLC6A3* VNTR polymorphism is located in the 3′ untranslated region of this gene; hence, it does not affect any structural or functional aspects of the transporter protein. However, Comings[106] has argued on the basis of molecular genetic research of polymorphisms of other genes,[107] that the different sizes of polymorphic alleles may nonetheless contribute to the regulation of gene expression. Consistent with this hypothesis, it has been reported that carriers of 2 copies of the 10-repeat allele of the *SLC6A3* gene have a lower availability of the transporter.[108] However, other studies have reported the opposite.[109] These discrepancies may be explained by differences in the demographic and clinical characteristics of the study samples and warrant further investigation to resolve them. Of particular interest, developmental differences in the level of expression of carriers of different alleles of the dopamine transporter requires further study. Evidence for a more general effect of the *SLC6A3* VNTR polymorphism on transcriptional activity has been reported.[100] Thus, how and when this polymorphism is involved in the modulation of the expression of the dopamine transporter or other neural pathways, including the mesocorticolimbic and nigrostriatal pathways, remains a critical question. Alternatively, this polymorphism may be completely silent but is in linkage disequilibrium with an unknown functional polymorphism. These 2 hypotheses need to be further explored by identifying other polymorphisms and testing them to identify their specific effect(s) on dopamine neurotransmission.

The *DRD4* 7-repeat allele has been linked to ADHD in many studies, but there have also been more recent studies refuting such an association. Given that most studies were subject to meta-analyses and the association between the polymorphism and ADHD remained robust, it is very likely that the association between *DRD4* and ADHD is real. In several cases, nonreplication may be due to sample sizes that are insufficient to rule out the involvement of *DRD4* or to heterogeneity in clinical characteristics of the patients studied. It is unclear whether the *DRD4* VNTR polymorphism has any effect on the structure or the function of the receptor. Indeed, Asghari et al[111] found that the sensitivity to dopamine of the 7-repeat allele form of the receptor was half that of the 2- and 4-repeat variants. However, several others report no significant impact of the VNTR variants on the function of the *DRD4* receptor.[112–114] Nonetheless, given that *DRD4* concentrations are high in key neuroanatomical areas implicated in ADHD, and given that the VNTR polymorphism or other polymorphisms in its vicinity could conceivably contribute to the "dopamine deficit" theories of the disorder, it is likely that the gene has a significant role in perpetrating its symptoms.

Genes discussed in this paper have been implicated in other disorders involving dopaminergic dysfunction. Family studies (e.g., Biederman et al[115]) have demonstrated a high comorbidity of ADHD and Tourette's syndrome, as well as conduct, oppositional defiant, mood, anxiety and other psychiatric disorders. Most likely, these disorders, including ADHD, involve subtle anomalies within similar circuits. It is therefore possible that the observed association between ADHD and either of the 2 genes is driven by the presence of these comorbid disorders. Studies correlating the variation in phenotypic expression, both for the comorbid symptoms as well as for other aspects of the clinical variability of ADHD (therapeutic response to psychostimulant drugs, hyperlocomotion, impulsivity and inattention considered as dimensions), will be very important in the future and may lead to a better nosological dissection of this complex disorder.

There is no question that ADHD is a polygenic disor-

der. No single gene has been found to account for more than 5% of the phenotypic variance of ADHD. Furthermore, heritability studies have attributed roughly 80% of the symptoms of ADHD to genetic factors, implying that the disorder is unlikely to be caused by a single gene. Finally, and most importantly, ADHD manifests as a wide spectrum of oftentimes varying symptoms, and it is doubtful that any one gene accounts for them all. Therefore, it is plausible that allelic variants in several different genes should characterize the disorder. At least 20 genes of small effect have been studied so far.[10] Compelling evidence implicates 2 of these genes, but more work is required to further confirm the role of other genes that may have less of an effect than *SLC6A3* and *DRD4*. Increasing sample sizes, using family-based association studies and controlling for the effect of the 2 major contributors identified up to now (*DRD4* and *SLC6A3*) may greatly help in clarifying the role of these other genes or in identifying new genetic risk factors that have not been previously studied.

ADHD is one of the rare psychiatric conditions where specific environmental factors have been implicated with relative confidence.[116–118] Assessing these risk factors in patients in genetic studies and controlling the effect of these risk factors while analyzing the genetics may help to better define the role of genes in ADHD and, possibly, to identify the mechanisms of interaction between genetic and environmental factors.

In conclusion, molecular genetic studies of ADHD are faced with the problem of heterogeneity that defines the disorder on a number of levels.[119] A careful phenotyping of children with ADHD on several dimensions, including symptom and neuropsychological profiles, comorbid conditions and therapeutic response to psychostimulant drugs, will be essential to guarantee advances in the genetic and, possibly, nosological dissection of this disorder. Future studies of the genetics of ADHD must also be sensitive to the polygenic nature of the disorder. Studies of additive gene effects, for example, may provide greater insight into the effects of individual genes. To date, roughly 20 genes have been analyzed in any single ADHD population, and it is likely for the reasons described above that there are many more loci involved. Gaining a greater insight into the true genetic makeup of ADHD will require much larger samples than are currently being studied, careful selection of the control population as well as a more accurate conception of the disorder.

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